"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD AND UV-SPECROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF BAMBUTEROL HYDROCHLORIDE AND MONTELUKAST SODIUM IN COMBINED DOSAGE FORM"

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CERTIFICATE

This is to certify that the Dissertation entitled **"Development and Validation of RP-HPLC method and UV -Spectrophotometric Simultaneous equation method of Bambuterol Hydrochloride and Montelukast Sodium in combined dosage form",** in combined dosage form" by **Mr. G. Velmurugan** In the department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai - 625 020, in partial fulfillment of the requirements for the Degree of Master of Pharmacy in Pharmaceutical Chemistry under my guidance and supervision During the academic year 2009-2010

This dissertation is forwarded to The Controller of Examination, The Tamilnadu Dr.MGR Medical University,Chennai.

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Date :

GENERAL INTRODUCTION

Analytical Chemistry deals with methods for determining the chemical composition of samples of matter. A quantitative method yield information about the identity of atomic or molecular species or the functional groups in the sample, a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

In Analytical Chemistry it is to prime importance to gain information about the qualitative and quantitative composition of substance and chemical species, that is to find out what a substance is composed and exactly how much. The goal of chemical analysis is to provide information about the composition of a sample of matter. In instrumental analysis, a physical property of a substance is measured to determined its chemical composition.[1]

1.1. CLASSIFICATION OF ANALYTICAL METHODS

Analytical methods are often classified as being either classical or instrumental. This classification is largely historical with classical methods, sometimes called wet chemical methods preceding instrumental methods by a century or more.

Classical Method

In the early years of chemistry, most analysis were carried out by separating the components of interest (the analytes) in a sample by precipitation, extraction, or distillation. For qualitative analysis, the separated components were then treated with reagents that yielded products that could be recognized by their colours, their boiling or melting points, their solubilities in a series of solvents, or their refractive indexes. For quantitative analysis, the amount of analyte was determined by gravimetric or by titrimetric measurements. In

gravimetric measurements, the mass of the analyte or some compound produced form the analyte was determined. In titrimetric procedure, the volume or mass of a standard reagent required to react completely with the analyte was measured.

Instrumental Method

Early in the twentieth century, chemists began to exploit phenomena other than those used for classical methods for solving analytical problems. Thus physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass-to charge ratio, and fluorescence began to be used for quantitative analysis of a variety of inorganic, organic and bio-chemical analytes. Furthermore, highly efficient chromatographic and electrophotometric techniques began to replace distillation, extraction, and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental method of analysis.[2]

The instrumental technique can be categorized into following types :-

A. Spectrophotometric technique :

1. Colorimetry
2. UV – Visible Spectrophotometry
3. Fluorescence and Phosphorescence spectrometry.
4. Atomic spectrometry
5. Infrared spectrometry
6. X-ray diffraction Method
7. Nuclear magnetic resonance spectrometry.
8. Electron spin resonance spectrometry.
9. Turbidimetry
10. Nephlomery etc.

B.Electrochemical Technique :

1. Conductometry
2. Potentiometry
3. Coulometry
4. Voltametry
5. Electro gravimetry

C. Chromatographic techniques :

1. Thin layer chromatography.
2. Gas chromatography
3. Super critical fluid chromatography
4. High performance liquid chromatography

D. Miscellaneous techniques :

1. Thermal analysis
2. Mass spectrometry
3. Kinetic technique

E. Hyphenated techniques :

1. LC – MS
2. LC – NMR
3. GC – MS

Table – 1

Classification of Analytical Methods

Characteristic Properties	Instrumental Methods
Emission of radiation	Emission spectroscopy (X-ray, UV, visible electron fluorescence, phosphorescence, and luminescence.
Absorption of radiation	Spectrophotometric and photometry (X-ray, UV, visible, IR), nuclear magnetic resonance and electron spin resonance spectroscopy.
Scattering of radiation	Turbidimetry; nephelometry, Raman spectroscopy
Refraction of radiation	Refractrometry; interferometry
Diffraction of radiation	X-ray and electron diffraction methods

Rotation of radiation	Polarimetry, optical dispersion; circular dichrosim
Electrical potential	Potentiometry; chronopotentiometry
Electrical charge	Coulometry
Electrical current	Amperometry; polarography
Electrical resistance	Conductometry
Mass	Gravimetry
Mass-to-charge ratio	Mass spectrometry
Thermal characteristics	Thermal gravimetry and titrimetry, differential scanning colorimetry; differential thermal analysies; thermal conductometric methods.
Radioactivity	Activation and isotope dilution methods

2.SPECTROSCOPY

Spectroscopy is a general term for the science that deals with the interaction of various types of radiation with matter. Spectroscopy and spectroscopic methods refer to the measurement of the intensity of radiation with a photometric transducer or other type of electronic device.

Analytical application of the absorption of radiation by matter can be either qualitative or quantitative. The qualitative and quantitative application of absorption spectrometry depend on the fact –

- A given molecular species absorbs radiation only in specific regions of the spectrum where the radiation has the energy required to raise the molecules to some excited state.

A display of absorption versus wavelength (or frequency) is called an absorption spectrum of that molecular species and services as a fingerprint for identification.[3]

2.1. UV SPECTROPHOTOMETRY

The technique of UV spectrophotometry is one of the most frequently employed in pharmaceutical analysis. It involves the measurement of the amount of UV (190-380nm) or visible (380-800nm) radiations absorbed by a substance in solution.

Molecular absorption in the ultraviolet (UV) and visible region of the spectrum is dependent on the electronic structure of the molecule. Absorption of energy is quantized, resulting in the elevation of electrons from orbitals in the ground state to higher energy orbital spin an excited state. For many electronic structures, the absorption does not occur in the readily accessible portion of the UV region. In practice, UV spectrometry is normally limited to conjugated systems [4]

Molecular absorption spectroscopy is based on the measurement of the transmittance (T) or the absorbance (A) of solutions contained in transparent cells having a path length of (b) cm. ordinarily, the concentration (c) of a absorbing analyte is linearly related to absorbance as represented by the equation.

$$A = -\log T = \log P_o/p = \in bc$$

This equation is a mathematical representation of Beer's law. [2]

Instruments which measure the ratio, or a function of the ratio, of the intensity of two beams of light in the UV region are called UV spectrophotometers. Absorption of light in both the UV and visible region of the electromagnetic spectrum occurs when the energy of light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions.

3. CHROMATOGRAPHY

Chromatography encompasses a diverse and important group of methods that permit the scientist to separate closely related components of complex mixtures, many of these separations are impossible by other means. In all chromatographic separations the sample is transported in a mobile phase, which may be a gas, a liquid, or a supercritical fluid. This mobile phase is then forced through an immiscible stationary phase, which is fixed in place in a column or on a solid surface. The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phase to varying degrees. Those components that are strongly retained by the stationary phase move only slowly with the flow of mobile phase. In contrast, components that are weakly held by the stationary phase travel rapidly. As a consequence of these differences in mobility, sample components separate into discrete hands, or zones, that can be analyzed qualitatively and / or quantitatively.

Chromatography can be defined as chemical separation technique based on the differential distribution of the constituents of a mixture between two phases, one of which moves relative to the other.

A fundamental classification of chromatographic methods is based upon the types of mobile and stationary phases and the kinds of equilibria involved in the transfer of solutes between phases. There are three general categories of chromatography, liquid chromatography, gas chromatography and supercritical chromatography. As the names imply, the mobile phases in the three techniques are liquids, gases and supercritical fluids respectively. [2]

Table 2

Classification of Chromatography

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid Chromatography (LC) (mobile phase liquid)	Liquid – liquid, or partition	Liquid absorbed on solid	Partition between immiscible liquids
	Liquid- bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid – solid- adsorption	Solid	Adsorption
	Ion-exchange resin	Ion-exchange resin	Ion exchange
	Size exchange	Liquid in interstices of a polymetric solid	Partition/sieving
Gas Chromatography (GC) (mobile phase gas)	Gas- liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas – bonded phase	Organic species boned to a solid surface	Partition between liquid and bonded surface
	Gas – solid	Solid	Adsorption
		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

3.1LIQUID CHROMATOGRAPHY :

Liquid chromatography is a method of chromatographic separation based on the difference in the distribution of species between two non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column.

Liquid chromatography is mainly based on mechanisms of adsorption, mass distribution, ion exchange, size exclusion or stereo chemical interaction. [5]

3.2 HIGH – PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC) is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity, and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. The rapid growth of the HPLC has been facilitated by the development of reliable, moderate priced instrumentation and efficient columns. Separation efficiencies achievable today are five to ten times greater than those available in the early.

Depending upon the mobile phase HPLC method can be classified into following types

1. Liquid – liquid chromatography (LLC)
2. Liquid – solid chromatography (LSC)
3. Ion – exchange chromatography
4. Size – exclusion chromatography

Liquid – solid chromatography often called the adsorption chromatography and liquid- liquid chromatography is termed partition chromatography, LLC can be divided into normal or reversed phase chromatography.

Liquid – solid chromatography or adsorption chromatography implies high surface area particles that adsorb the solute molecules. Usually a polar solid such as silica gel, alumina (Al_2O_3) or porous glass beads and non-polar mobile phase such as heptanes, octane, or chloroform are used in adsorption chromatography, the differences in affinity of the solutes for the surface of the stationary phase account for the separations achieved. The compound has little affinity for the stationary phase and hence elutes quickly. The compound has a much higher affinity and is retained longer in the system. Generally, in adsorption HPLC, compounds elute in the reverse order of their polarities.

In liquid-liquid or partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases

determines the separation. The stationary phase can be either polar or non-polar. If the stationary phase is polar and the mobile phase is non-polar, it is called normal –phase partition chromatography. If the opposite case holds, it is called reversed phase partition chromatography. In the normal phase mode, the polar molecules partition preferentially into the stationary phase and are retained longer than non-polar compounds. In reversed phase partition chromatography, the opposite behavior is observed.

Ion-exchange chromatography uses stationary phases that can exchange cationic or anionic species with the mobile phase. In this mode, a reversible exchange of ions takes place between the stationary ion-exchange phase and the liquid mobile phase. Separations are achieved due to the differences in strength of electrostatic interactions of the solutes with the stationary phase.

Gel-permeation or size-exclusion chromatography are methods based on separation according to the size of the molecules. In this type of chromatography, the materials used for the stationary phases contain pores of certain sizes. Molecules that are too large are excluded from the pores while smaller molecules enter into the pores. The larger molecules remain in the following mobile phase and are eluted first. The smaller molecules, while in the pores, do not travel as fast and are eluted last. [6]

3.3 REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Reverse phase chromatography refers to the use of a polar eluent with a non polar stationary phase in contrast to normal phase chromatography, where a polar stationary phase is employed with anon – polar mobile phase.

Reverse phase chromatography is widely used due to the following advantages

- Many compounds such as biologically active substances have limited solubility in non polar solvents that are employed in normal phase chromatography.
- Ionic or highly polar compounds have high heats of adsorption on straight silica or alumina columns and therefore can elute as a tailing peaks.
- Column deactivation from polar modifiers is a problem in liquid solid chromatography which frequently leads to irreducibility in chromatography systems.
- Ionic compounds can be chromatographed via ion exchange chromatography. This mode of chromatography is tedious because precise control of variables such as pH and ionic strength is required for reproducible chromatography.

Reverse phase mobile phases

The mobile phase in **RPHPLC,** however, has a great influence on the retention of the solutes and the separation of component mixtures.

The primary constituent of reverse phase-mobile phase is water. Water miscible solvents such as methanol, ethanol, acetonitrile, dioxin, tetrahydrofuran are added to adjust the polarity of the mobile phase. The water should be high quality, either distilled or demineralised. The most widely used organic modifiers are methanol, acetonitrile and tetrahydrofuran. Methanol and acetonitrile have comparable polarities but the latter is an aportic solvent. This factor may be important if hydrogen bonding plays a significant role in the separation. When organic salts and ionic surfactants are used, the mobile phase should be filtered before use since these additives frequently contain a significant amount of water insoluble contaminants that may damage the column. Reverse phase mobile are

generally noninflammable due to high water content. Degassing is quite important with reverse phase mobile phases.

Selection of Mobile Phase

Flowing points are considered for the selection of a mobile phase.

1. Viscosity.
2. Compressibility
3. Refractive index
4. UV cutoff
5. Polarity
6. Vapour pressure
7. Flash point.

Reverse Phase HPLC Detectors

Detectors for HPLC fall into general categories. Differential detectors or bulk property detectors provide a differential measurement of a bulk property that is possessed by both the solute and the mobile phase. These detectors are generally nonspecific and respond to a wide range of compounds. eg. Refractive index detectors. The solute property or selective detectors measures a property of the sample which is not possessed by the mobile phase, eg. Ultraviolet and fluorescence detectors.

4. VALIDATION

Validation is defined by different agencies.

USFDA : According to this "Validation is the process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.

WHO : Defines Validation as an action of providing any procedure, process, equipment, material, activity or system actually leads to the expected results.

EUROPEAN COMMITTEE: Defines Validation as an action of providing in accordance with the principles of GMP that any procedure, process, material, activity or system actually lead to expected results.

This process consists of establishment of the performance characteristics and the limitations of the method.

Objective

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose & it gives the assurance that the drug product have the identify strength, quality and purity.

a) Quality, safety and efficacy must be designed and built into the product.
b) Each step of the manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specification.

When validation is needed

- For the introducing a new method in routine use.
- Whenever change in the synthesis of drug substance
- Whenever change in the composition of the finished product. [7]

Requirement for validation

- Calibration report of instruments
- A targeted goal to be achieved
- Protocols
- A procedure of validation that is validated
- All the documents of prevalidated documentation procedure.
- Reviewing of all the predetermined intervals or events.
- Authentication of all the above by individuals who are considered to be fir for authentication.

Whatever is not validated is considered to be invalid or unfit for use [8]

Types of analytical procedures to be validated

Four most common types of analytical procedures to be validated

- Identification test
- Quantitative test for impurities content
- Limit tests for the control of impurities
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product. [9]

Purpose for validation

- Enable scientists to communicate scientifically and effectively on technical matters.
- Setting standards of evaluation procedures for checking compliance and taking remedial measures.

- Reduction in cost associated with process sampling and testing. The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes thus providing indirect cost saving from reduced testing or re-testing and elimination of product rejection.
- As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples, the validation thus shall provide adequacy and reliability of a system or a procedure to meet the pre-determined criteria attributes providing high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.
- Retrospective validation is useful for trend comparison of results compliance to cGMP/cGLP.
- Closer interaction with pharmacopoeial forum to address analytical problems.
- International pharmacopoeial harmonization particularly in respect of impurities determination and their limits.
- For taking appropriate action in case of non-compliance.

Selection of analytical method

First stage in the selection or development of method is to establish what is to be measured and how accurately it should be measured. Unless one has series of method at hand to assess quality of the product, validation, programme may have limited validity. The selected method must have the following parameters.

1. As simple as possible
2. Most specific
3. Most productive, economical and convenient

4. As accurate and precise as required.
5. Multiple source of key components (reagents, columns, TLC plates) should be avoided.
6. To be fully optimized before transfer for validation of its characteristics such as accuracy precision, sensitivity, ruggedness etc. [10]

4.1 ANALYTICAL METHOD VALIDATION

Method validation is a process of establishing performance characteristics and limitations of a method and identification of the influences which may change the characteristics and to what extent. It is also used for solving a particular analytical problem.

Validated analytical test methods are required by good manufacturing practice (GMP) regulations for products that have been authorized for sale and almost certainly for late-stage trial clinical material. Also, some methods used during the pre-clinical phase of drug development under good laboratory practice (GLP) regulations may also require validation.

"Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications".

Analytical validation is the core stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do.

It is the process of proving that an analytical method is acceptable for its intended purpose. For pharmaceutical methods, guidelines from the united states Pharamacopoeia (USP), international conference on Harmonization (ICH) and the food and drug administration (FDA) provide a framework for performing such validations.

The purpose of method validation is to demonstrate that the established method is “fit for the purpose”. This means that the method, as used by the laboratory generating the data, will provide data that meets the criteria set in the planning phase. There is not a single accepted procedure for conducting a method validation. Much of the method validation and development are performed in an interative manner, with adjustments or imporovements to the method made as dictated by the data. The analyst’s primary objective is to select an approach that will demonstrate a true validation while working in a situation with defined limitations, such as cost and time. All new methods developed are validated.

Assay Category I

Analytical method for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

Assay Category II

Analytical method for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These methods include quantitative assays and limit tests.

Assay Category III

Analytical method for determination of performance characteristics. (E.g. dissolution, drug release profile).

Assay Category IV

Identification tests

For each assay category, different analytical information is needed. Data elements that is normally required for each of the categories of assays given in the following table.

Table 3

Data elements required for assay validation as per USP

Parameters	Assay Category I	Assay Category II		Assay Category III	Assay Category IV
		Quantitative	Limit Tests		
Specificity or selectivity	Yes	Yes	*	*	No
Accuracy	Yes	Yes	No	Yes	No
Precision	Yes	Yes	Yes	*	Yes
Detection Limit	No	No	Yes	*	No
Quantitation Limit	No	Yes	No	*	No
Linearity	Yes	Yes	No	*	No
Range	Yes	Yes	*	*	No

Analytical method validation parameters

- Accuracy
- Precision
- Specificity
- Limit of Detection
- Limit of Quantitation
- Linearity and Range

- Ruggedness
- Robustness
- System suitability

Method validation is completed to ensure that an analytical methodology is accurate, specific, reproducible and rugged over the specified range that an analyte will be analyzed. Method validation provides an assurance of reliability during normal use, and is sometime referred to as “the process of providing documented evidence that the method does what it is intended to do.” Regulated laboratories must perform method validation in order to be in compliance with FDA regulation.

Accuracy

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the value which is accepted either as a conventional, true value or an accepted reference value and the value found. It is measure as the percent of analyte recovered by assay.

Precision

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. According to the ICH, precision should be performed at three different levels: repeatability, intermediated precision, and reproducibility.

1. Repeatability is the results of the method operating over a short time interval under the same conditions (inter-assay precision). It should be determined from a minimum of nine determinations covering the specified range of the procedure (for example, three levels, three repetitions each) or from a minimum of six determinations at 100% of the test or target concentration.

2. Intermediate precision is the results from within lab variations due to random events such as different days, analysts, equipment, etc. in determining intermediate precision, experimental design should be employed so that the effects (if any) of the individual variables can be monitored.
3. Reproducibility refers to the results of collaborative studies between laboratories. Documentation in support of precision studies should include the standard deviation, relative standard deviation, coefficient of variation, and the confidence interval.

Specificity

Specificity is the ability to measure accurately and specifically and analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to single component only.

Limit of Detection

The limit of detection (LOD) is defined as the lowest concentration of an anlayte in a sample that can be detected, not quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value. It is expressed as a concentration at a specified signal-to-noise ratio, usually two-or three-to-one.

LOD's may also be calculated based on the standard deviation of the response (σ) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula:

$$LOD = 3.3(\sigma/S)$$

Limit of Quantitation

The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Like LOD, LOQ is expressed as a concentration, with the precision and accuracy of the measurement also reported. Sometimes a signal-to-noise ratio of ten-to-one is used to determine LOQ. This signal-to-noise ratio is a good rule of thumb, but it should be remembered that the determination of LOQ is a compromise between the concentration and the required precision and accuracy. That is, as the LOQ concentration level decreases, the precision increases. If better precision is required, a higher concentration must be reported for LOQ.

The calculation method is again based on the standard deviation of the response (σ) and the slope of the calibration curve (S) according to the formula:

$$LOQ = 10(\sigma/S)$$

Linearity and Range

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line. Range is the interval between the upper and lower levels of analyte (inclusive) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results obtained by the method.

Ruggedness

Ruggedness, according to the USP, is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. These conditions include different laboratories, analysts, instruments, reagents, days, etc.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. The robustness of a method is evaluated by varying method parameters such as percent organic, pH, ionic strength, temperature, etc., and determining the effect (if any) on the results of the method.

System Suitability

According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment , electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole.

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility are determined and compared against the specifications set for the method. These parameters are measured during the analysis of a system suitability “sample” that is a mixture of main components and expected by-products. [9, 11, 12]

4.2. MERITS AND DEMERITS OF ANALYTICAL METHOD VALIDATION

Merits

- Reliability of analytical results and assurance of quality product.
- Performance capability of the method can be confirmed by analysts using the method.
- Awareness about importance of protocols for validation work.
- Motivation for improvement in quality of work.
- Provides opportunity for training to QC staff.
- Helps in scientific communication on technical matters.

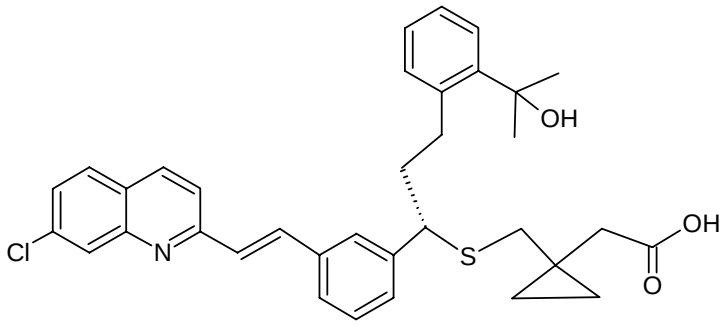
Demerits

- Increasing cost.
- Need for experienced personnel. [13]

DRUG PROFILE [14, 15, 16]

MONTELUKAST SODIUM

Molecular structure:



Chemical Name : [R-(E)]-1-(((1-(3-(2-(7-chloro-2-quinolinyl) ethenyl) Phenyl)-3-(2-(1-hydroxy-1-methyl ethyl)phenyl) Propyl) thio)methyl) cyclopropane acetic acid.

Molecular Formula : $C_{35}H_{36}ClNO_3S$

Molecular Weight : 586.18

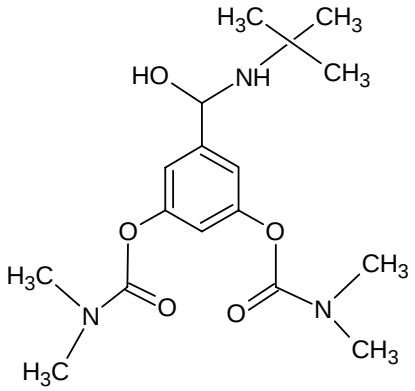
Appearance : White or almost white powder

Solubility : Soluble in methanol, Insoluble in 0.1 N HCl, Partially soluble in distilled water.

Action and use : Anti-ashmatic drug.

BAMBUTEROL HYDROCHLORIDE [14, 15, 16, 17]

Molecular structure:



Chemical Name : [3[2(tert-butyl amino)-1-hydroxy ethyl]-5-(dimethyl carbamoyl oxy)-phenyl] N,N dimethyl carbamate.

Molecular Formula : $C_{18}H_{29}N_3O_5$

Molecular weight : 367.440

Appearance : White or almost white powder

Solubility : soluble in methanol

Action and use : Antiashmatic drug.

REVIEW OF LITERATURE

Alsarra et al., developed a stability-indicating HPLC method for the determination of Montelukast in tablets and human plasma and its application to pharmacokinetic and stability studies. The intra day and interday precisions showed coefficients of variations ranged from 5.87% to 9.60% and from 2.13% to 6.18% at three different levels of concentrations.[18]

Radhakrishna et al., compared HPLC and derivative spectrophotometric methods for the simultaneous determination of Montelucast and Loratidine. HPLC separation was achieved with a symmetry C18 column and sodium phosphate buffer (pH 3.7): acetonitrile(20:80v/v) as eluent at a flow rate of 1.0 ml/min . UV detection was performed at 225 nm. In the UV second- order derivative spectrophotometry for the determination of Loratidine the zero-crossing technique was applied at 276.1 nm but for Montelucast peak amplitude at 359.7 nm (Tangent method) was used.[19]

Liu-L et al., developed a stereo selective HPLC with column switching for the determination of Montelucast and its enantiomer in human plasma.[20]

Alsarra et al., Developed a spectrofluorimetric determination of Montelukast in dosage forms and spiked human plasma. The highest fluorescence intensity was obtained in methanol at 390 nm using 340 nm for excitation.[21]

Amin RD et al., carried the determination of Montelukast-0476 in human plasma by HPLC. The method involves precipitation of protein and reversed-phase HPLC with fluorescence detection. The assay is linear in the range of 30-3000 ng/ml-1 of MK-0476 and the limit of detection is 5 ng/ml-1. The interday accuracy values at these concentrations are 94 and 104% respectively. The absolute recovery of MK-0476 is 99%.[22]

Alsarra et al., developed a voltammetric determination of Montelukast sodium in dosage forms and human plasma. It was studied using cyclic voltammetry, direct current (DCT) differential pulse polarography (DPP) and alternating current (ACT) Polarography. The mean percentage recovery (n=5) was 101.38+/- 3.85. The number of electrons

transferred in the reduction process could be accomplished and a proposal of the electrode reaction was proposed.[23]

Ibrahim A. Alsarra developed a stability-indicating high performance liquid chromatographic (HPLC) method has been developed and validated for the determination of montelukast in human plasma and in its pharmaceutical dosage from. The proposed method has been also applied for the determination of montelukast in the presence of its degradation product. Acetonitrile: potassium dihydrogen phosphate (0.05 M) adjusted to pH 3.5 ± 0.1 with phosphoric acid (70:30, % v/v) was used as the mobile phase at a flow rate of 2.0 ml/min using a Symmetry C18 column. The effluent was spectrophotometrically monitored at 345 nm. Peak area ratio of the drug to the internal standard (flufenamic acid) was used for the quantification of montelukast in plasma samples and the limit of quantification was 10 ng/ml and the limit of detection was 1.0 ng/ml. The intraday and interday precisions showed coefficients of variation ranged from 5.87% to 9.60% and from 2.13% to 6.18% at three different levels of concentrations. [24]

Shamkant S. Patil, Shinde Atul et al., determination of Three simple, precise and economical UV methods have been developed for the estimation of Montelukast in bulk and pharmaceutical formulations. Montelukast has the absorbance maxima at 359nm (Method A), and in the first order derivative spectra, showed zero crossing at 359nm, with a sharp peak at 340.5nm when n=1 (Method B), Method C applied was Area Under Curve (AUC). For analysis of Montelukast the wavelength range selected was 350-370 nm. Drug followed the Beer's Lamberts range of 5-40 μg/ml for the Method A, B C. Results of analysis were validated statistically and by recovery studies and were found to be satisfactory.[25]

Lin Zhu, Likun Chen, BinGuo et al., A chiral chromatography/tandem mass spectrometry bioanalytical method for the determination of bambuterol and terbutaline and their enantiomers in rat plasma was developed. The method employed protein precipitation method for sample extraction. A Chirobiotic T Spherical column was used for chiral separation using a polar organic mobile phase consisting of methanol and 0.2mmol/L ammonium formate. The analytes were detected by a tandem mass spectrometer operated in positive ion mode. The (S)- and (R)-isomers of bambuterol were resolved chromatographically with retention times of 23.42 and 20.89 min, respectively. The (S)- and (R)-isomers of terbutaline was 18.25min and 16.08min, respectively. The analytical run time

was 30 min. The lower limit of quantitation (LLOQ) was 5ng/mL for both enantiomers. The polar organic mode chiral chromatography provided a specific, rugged method for the chiral analysis of bambuterol in biological fluids.[26]

C. Bosch Ojeda, F. Sanchez Rojas et al., Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Thus, the information content of a spectrum is presented in a potentially more useful form, offering a convenient solution to a number of analytical problems, such as resolution of multi-component systems, removal of sample turbidity, matrix background and enhancement of spectral details. Derivative spectrophotometry is now a reasonably priced standard feature of modern micro-computerized UV/Vis spectrophotometry.[27]

Rosa Ventura, Lúcia Damasceno et al, Acomprehensive gas chromatographic–mass spectrometric (GC–MS) procedure for detection in urine ofb2-agonists having different alkyl or phenylalkyl chains at the nitrogen atom is described. The method is based on an enzymatic hydrolysis with b-glucuronidase from *Helix pomatia*, followed by a solid-phase extraction procedure using Bond Elut Certify columns. The influence of urinepHin the extraction recovery has been studied andpH9.5was found to give best recovery and cleaner extracts. After pH adjustment, the sample was applied to the pre-conditioned cartridges and after a washing step, the b2-agonists were eluted with a mixture of chloroform and isopropanol (80:20, v/v) containing 2% ammonia. The residues were derivatised with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA), and analysed by GC–MS.Avalidation procedure for qualitative analysis of b2-agonists in urine was performed.[28]

Nitesh K. Patel, Gunta Subbaiah et al., A rapid liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS=MS) method was developed for the determination of montelukast in human plasma. The extraction of montelukast from plasma(300 mL) involved protein precipitation. Quantitation was performed using LC-ESI-MS=MS, operating in the positive ion and selective reaction monitoring (SRM) mode. The total chromatographic run time for the analysis was 1.5 min. A linear dynamic range was

established from 5 to 800 ng mL_1 for montelukast. The method was fully validated especially with regard to real subject sample analysis.[29]

D. Vijaya Bharathi, Kishore Kumar et al., A highly sensitive, rapid assay method has been developed and validated for the estimation of montelukast (MTK) in human plasma with liquid chromatography coupled to tandem mass spectrometry with electro spray ionization in the positive-ion mode. Liquid–liquid extraction was used to extract MTK and amlodipine (internal standard, IS) from human plasma.Chromatographic separation was achieved with 10mM ammonium acetate (pH 6.4): acetonitrile (15:85, v/v) at a flow rate of0.50 mL/min on a Discovery HS C18 column with a total run time of 3.5min. The MS/MS ion transitions monitored were 586.10 → 422.10 for MTK and 409.20 → 238.30 for IS. Method validation and clinical sample analysis were performed as per FDA guidelines and the results met the acceptance criteria. The lower limit of quantitation achieved was 0.25 ng/mL and linearity was observed from 0.25 to 800 ng/mL. The intra-day and inter-day precisions were 5.97–8.33 and 7.09–10.13%, respectively. [30]

M. Saeed Arayne, Najma Sultana et al., A simple ultraviolet spectrophotometric method for the estimation of montelukast in methanol has been devised and been compared with the existing pharmacopoeial RP-HPLC method for estimation of the drug. The limit of detection of montelukast at 283 nm was 75.2 ng/mL. The calibration was linear in the range of 3–45 μg/mL. Analytical parameters such as stability, selectivity, accuracy and precision have been establishedfor the method in MONAKA® tablets and in human serum and evaluated statistically to assess the application of the method. The method was validated under the ICH and USP guidelines and found to comprise the advantages for simplicity, stability, sensitivity, reproducibility and accuracy for using as an alternate to the existingnon-spectrophotometric methods for the routine analysis of the drug in pharmaceutical formulations and in pharmaceutical investigations involving montelukast.[31]

Hisao Ochiai, Naotaka Uchiyama et al., MK-0476 (montelukast sodium) is a potent and selective cysteinyl leukotriene receptor antagonist that is being investigated in the treatment of asthma. A simple and sensitive method for the determination of MK-0476 in human plasma was developed using column-switching high-performance liquid chromatography (HPLC) with fluorescence detection. A plasma sample was injected directly

onto the HPLC system consisting of a pre-column (Capcell pak MF) and an analytical column (Capcell pak C18) which were connected with a six-port switching valve. The column eluate was monitored with a fluorescence detector (excitation at 350 nm; emission at 400 nm). The calibration curve was linear in a concentration range 21 of 1–500 ng ml for MK-0476 in human plasma. The intra-day coefficients of variation of all concentrations within the range was less than 9.2%, and the intra-day accuracy values were between 97.2 and 114.6%. This method was used to measure the plasma concentration of MK-0476 following oral administration of the drug in humans.[32]

Martin Josefsson, Alma Sabanovic et al, Alternative strategies for sample preparation of human blood samples were evaluated including protein precipitation (PP) and solid phase extraction (SPE) on Waters Oasis® polymeric columns. Gradient chromatography within 15 min was performed on a Hypersil Polar-RP column combined with a Sciex API 2000 triple quadrupol instrument equipped with an electro-spray interface. Beta-agonists and beta-antagonists available on the Swedish market were included in the study. A combination of zinc sulphate and ethanol was found effective for PP. A clear supernatant was achieved that either could be injected directly on the LC–MS–MS system for analysis or transferred to a SPE column for further extraction and analyte concentration. Retention on the hydrophilic–lipophilic balanced sorbent HLB as well as the mixed mode cationic MCX and anionic MAX sorbents were investigated.[33]

Sameer Al-Rawithi, Sulaiman Al-Gazlan et al., This study describes an expedient assay for the analysis of the asthma medication, montelukast sodium (Singulair,MK-0476), in human plasma samples. After a simple extraction of the plasma, the drug and internal standard, quinine bisulfate, were measured by HPLC. The chromatographic system consisted of a single pump, a refrigerated autosampler, a C 4-mm particle size radial compression cartridge at 408C and a fluorescence detector with the excitation and emission 8 wavelengths set at 350 and 400 nm, respectively. The mobile phase which was delivered at 1.0 ml/min, was prepared by adding 200 ml of 0.025 *M* sodium acetate, pH adjusted to 4.0 with acetic acid, to 800 ml of acetonitrile, with 50 ml triethylamine. With a run time of only 10 min per sample, this assay had an overall recovery of .97% with a detection limit of 1 ng/ ml. The inter- and intra-run relative standard deviations at 0.05, 0.2 and 1.0 mg/ml were all ,9.2%,

while the analytical recovery at the same concentrations were within 7.7% of the amount added.[34]

Lucia Damascenoa, Rosa Ventura et al., A GC–MS procedure for the detection of different b-agonists in urine samples based on two consecutive derivatization steps is described. The derivatization procedure is based on the consecutive formation of cyclic methylboronate derivatives followed by a second derivatization step with MSTFA on the same extract, forming TMS derivatives. Injections in the GC–MS system may be carried out after each one of the derivatization steps, obtaining enough information for unambiguous identification. Limits of detection for the two derivatization steps ranged from 0.5 to 5 ng/ ml. This procedure was tested with the b-agonists bambuterol, clenbuterol, fenoterol, formoterol, salbutamol, salmeterol, a-hydroxy-salmeterol and terbutaline.[35]

W. Van Thuyne, P. Van Eenoo et al., A selective and sensitive screening method for the detection of prohibited narcotic and stimulating agents in doping control is described and validated. This method is suitable for the detection of all narcotic agents mentioned on the World Anti-Doping Agency (WADA) doping list in addition to numerous stimulants. The analytes are extracted from urine by a combined extraction procedure using CH2Cl2/MeOH (9/1, v/v) and *t*-butylmethyl ether as extraction solvents at pH 9.5 and 14, respectively. Prior to GC–MS analysis the obtained residues are combined and derivatised with MSTFA. The mass spectrometer is operated in the full scan mode in the range between *m*/*z* 40 and 550. The obtained limits of detection (LOD) for all components included in this extensive screening method are in the range 20–500 ng/ml, which is in compliance with the requirements set by WADA. Besides narcotic and stimulating agents, this method is also capable of detecting several agents with anti-estrogenic activity and some beta-agonists.[36]

Robert Papp, Pauline Luk et al., A rapid LC–MS/MS method was developed and partially validated for the quantitation of montelukast in spiked sheep plasma. A total run time of 1.5 min was achieved using a short monolithic column and employing a rapid gradient. Sample preparation involved protein precipitation with twofold acetonitrile by volume during which a deuterated internal standard (montelukast D-6) was incorporated. The MRM transitions for montelukast and the deuterated internal standard were 586/422 and 592/427, respectively. A linear dynamic range of 0.25–500 ng/mL with a correlation

coefficient of 0.9999 was achieved. Precision was below 5%at all levels except at the LOQ (0.36 ng/mL) which demonstrated an overall of R.S.D. of 8%. Post-column infusion experiments were performed with precipitated plasma matrix and showed minimal interference with the peaks of interest.[37]

Pattana Sripalakit, Bungon Kongthong et al., An analytical method based on high-performance liquid chromatographic (HPLC) was developed for the determination of montelukast in human plasma using mefenamic acid as an internal standard. After precipitation of plasma proteins with acetonitrile, chromatographic separation was carried out using a Zorbax Eclipse® XDB C8 (150mm×4.6mm i.d., 5_m) with mobile phase consisted of methanol–acetonitrile–0.04M disodium hydrogen orthophosphate (22:22:56, v/v, pH 4.9). The wavelengths of fluorescence detectionwere set at 350nm for excitation and 450nm for emission. The linearity was confirmed in the concentration range of 5–1000 ng/ml inhumanplasma. Intra- and inter-day accuracy determined from quality control samples were 101.50 and 107.24%, and 97.15 and 100.37%, respectively. Intra- and inter-day precision measured as coefficient of variation were ≤4.72 and ≤9.00%, respectively.[38]

Lida Liu, Haiyung Cheng et al., A steoreoselective high-performance liquid chromatographic method was developed for the quantification of montelukast (free acid of Singulair TM, or MK-0476), a potent and selective leukotriene D 4 (cysLTl) recptor antagonist, and it S-enantiomer (L-768,232). The method involves protein precipitation and fluorescence detection. Chromatographic separation of the enantiomers from endogenous components in plasma and chiral resolution of the enantiomers are achieved by using column switching HPLC and an :~-acid glycoprotein chiral column. The assay is linear in the range of 28.9-386 ng ml 1 of free acids of montelukast and L-768,232. The intraday precision (% relative standard deviation) values of this method were in the range of 2.5-9.1% for montelukast, and 2.4 6.8% for L-768,232, while the intraday accuracy values were in the range of 97-103% for montelukast and 96-104"/,, for L-768,232. The interday precision values of this method at 48.2 and 193 ng ml l were 5.3 and 3.6%, respectively, for montelukast, and 4.2 and 3.7°/,,, respectively, for L-768,232.[39]

Chester J. Kitchen, Amy Q. Wang et al., A simple, semi-automated, protein precipitation assay for the determination of montelukast (SINGULAIRTM, MK-0476) in

human plasma has been developed. Montelukast is a potent and selective antagonist of the cysteinyl leukotriene receptor used for the treatment of asthma. A Packard MultiPROBE†II EX is used to transfer 300 ml of plasma from sample, standard, and QC sample tubes to a microtiter plate (96-well). After addition of the internal standard by a repeating pipettor, a Tomtec QUADRA 96† adds 400 ml of acetonitrile to all plasma sample wells, simultaneously, in the microtiter plate. The Tomtec is also used to transfer the acetonitrile supernatant from the plasma protein precipitation step, batchwise, to another microtiter plate for analysis by HPLC with fluorescence detection. This assay has been validated and implemented for a clinical study of over 1300 plasma samples and is comparable to manual assays in the LLOQ (lower limit of quantitation, 3 ng/ml) and in stability. This is the first semi-automated protein precipitation assay published for the analysis of montelukast in human plasma and it results in significant time savings over the manual methods, both in sample preparation and in HPLC run time.[40]

A. Bartolinˇci´, V. Druˇskovic et al., Suitable HPLC methods for the direct separation of bambuterol and albuterol enantiomers were developed. The enantioseparation was tested on numerous commercial chiral HPLC columns. For bambuterol the most convenient separation was determined on amylose Chiralpak AD column, and for albuterol on vancomycine Chirobiotic V column. The mobile phase compositions were systematically studied to obtain the optimal chromatographic methods. Validation of methods in selected conditions shows that the chosen methods are selective and precise with linear response of detector for both pairs of enantiomers.[41]

Robin K. Harris , Paul Hodgkinson et al., Carbon-13 NMR spectra of the stable polymorphs of solid bambuterol hydrochloride (BHC) and terbutaline sulfate (TBS) are reported and the resonances assigned with the aid of solution-state spectra. A protocol is presented for quantification of BHC in a formulation in lactose, together with TBS, relative to a reference peak from magnesium stearate. This protocol compares the intensity of an aromatic signal of BHC with that of the main-chain methylene carbons of the stearate. It is shown that the limit of detection (LOD) of BHC in this system under the conditions described is 0.5% with an effective limit of quantification (LOQ) of 1.0%.[42]

Wenxia Luo, Lin Zhu et al., A chiral liquid chromatography–tandem mass spectrometry (LC–MS/MS) simultaneous stereoselective analysis of bambuterol and its active metabolite terbutaline enantiomers in Wistar rat plasma has been developed and validated. All analytes and the internal standard were extracted from rat plasma samples by liquid–liquid extraction, separated on macrocyclic glycopeptide teicoplanin column with mobile phase constituted of 20mM ammonium acetate solution–methanol (10:90, v/v) at a flow-rate of 0.4 mL/min. Detection was performed on an API 3000 tandem mass spectrometer with positive electrospray ionization in multiple reaction monitoring mode. The calibration curves in the range 1–800 ng/mL were linear and the accuracy for each analyte was within 8.0%. The intra- and inter-day precision as determined from quality control samples was less than 10.1%. The validated assay was successfully used to determine the enantiomers of bambuterol and terbutaline in rat plasma samples in the pharmacokinetic studies of rac-bambuterol.[43]

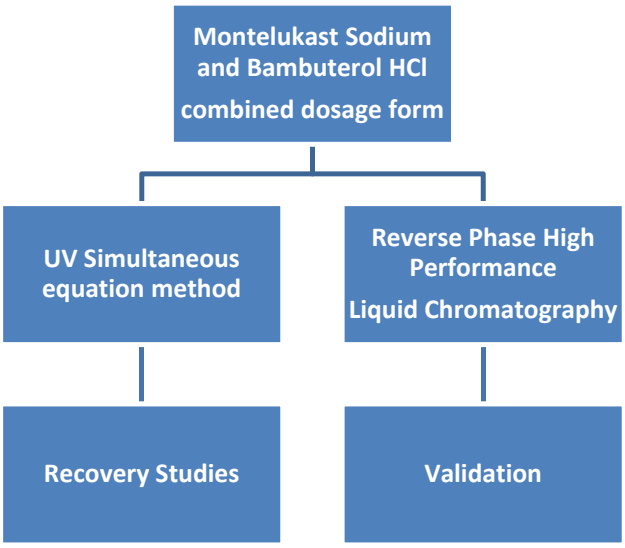
AIM OF THE WORK

The combination of an Anti-asthmatic drugs Montelukast Sodium and Bambuterol HCl are selected for analysis by UV spectrophotometric method and reverse phase high performance liquid chromatography.

It is estimated by simultaneous equation method as the λmax of the drugs are dissimilar and their absorbance ratio lies outside the range 0.1 to 2.

The next objective is to develop and validate a reverse phase high performance liquid chromatography which would be simple, rapid, efficient and reliable for the analysis of both the drugs in combined dosage form.

SCHEME OF THE WORK



REVERSE PHASE HIGH PERFOMANCE LIQUID CHROMATOGRAPHY AND VALIDATION IN ESTIMATION OF MONTELUKAST SODIUM AND BAMBUTEROL HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM.

Instruments:

- Shimadzu liquid chromatograph LC – 20 AT VP
- Mettler Toledo AG 285 Balance CP-225D
- DIGISUN-DI-707 pH meter
- Millipore filter (10.45/ μm)
- Whatman filter paper
- Sonicator

Reagents and Chemicals

- Acetonitrile
- HPLC grade water
- Buffer

REFERENCE STANDARDS:

Montelukast Sodium and Bambuterol HCl:

These two reference standards were obtained as gift samples from Ceeal analytical laboratory,Chennai. The authenticity and purity of the sample was certified by the same.

Sample Tablet brand used : Montek-plus

Label claim : Montelukast Sodium - 10 mg

Bambuterol HCl - 10 mg

METHOD DEVELOPMENT AND OPTIMIZATION:

SELECTION OF WAVELENGTH:

The known concentration of Montelukast Sodium and Bambuterol HCl were taken and dissolved in THF (Tetra hydro furan). The wavelength were tried from 200nm to 400nm and the Peaks of the drugs were showing fronting and tailing. The peak areas were also found to be minimum. Finally 230nm were selected for the analysis.

OPTIMIZATION OF CHROMATOGRAPHIC PARAMETAERS

(a) **SELECTION OF MODE OF OPERATION**:

As both the drugs were are polar in nature, a RP-HPLC method was Proposed.

(b) **SELECTION AND STANDARDISATION OF MOBILE PHASE:**

MONTEK-PLUS is a combination of Montelukast sodium 10mg and Bambuterol HCl 10mg. The method development of Montelukast sodium and Bambuterol HCl required adequate resolution of two drug peaks in the chromatogram.

DIFFERENT COMBINATIONS OF BUFFER AND SOLVENTS:

Buffer(potassium dihydrogen ortho phosphate pH:3) and acetonitrile (50:50)

Buffer(potassium dihydrogen ortho phosphate pH:3) and acetonitrile (20:80)

Buffer(potassium dihydrogen ortho phosphate pH:3) and acetonitrile (30:70)

Buffer(potassium dihydrogen ortho phosphate pH:3) and methanol and acetonitrile (40:30:30),finally add 2.5% v/v THF

Buffer(potassium dihydrogen ortho phosphate pH:3) and methanol and acetonitrile (45:20:35),finally add 5% v/v THF

Peaks of Montelukast sodium and Bambutero HCl were well resolved with solvent system Buffer (Potassium dihydrogen phosphate pH:6 with dil KoH) acetonitril.(60:40).

SELECTION OF FLOW RATE:

The Flow rate for Montelukast sodium and Bambuterol HCl were tried with 0.5ml, 1ml, 1.5ml and 2ml, the peaks of the drugs were showing fronting and tailing with 0.5ml and 2ml respectively and finally 2ml per minute was selected for the analysis.

PREPARATION OF BUFFER SOLUTION:

Buffer solution was prepared by using 6.8g of potassiumdi hydrogen phosphate dissolved in 1000ml of HPLC grade water, pH adjusted to 6 with dil KoH, filtered through 0.45µ nylon membrane and degassed.

PREPARATION OF MOBILE PHASE:

Mix the Buffer and acetonitrile in the ratio of 60:40 ,finally add 5% v/v THF and degass it. Filtered through 0.45µ membrane.

DILUENT

Mobile phase is used as diluent.

DETERMINATION OF RETENTION TIME:

(A) STANDARD SOLUTION OF MONTELUKAST SODIUM:

Accurately 104.6mg of Montelukast sodium was taken in a 100ml volumetric flask and dissolved in 10ml mobile phase, the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 100µg/ml of Montelukast sodium. 20µl of this solution was injected and chromatogram was obtained.

(B) STANDARD SOLUTION OF BAMBUTEROL HCL:

Accurately 100.4mg of Bambuterol HCl was taken in a 100ml volumetric flask and dissolved in 10ml mobile phase,the volume was adjusted to 100ml with mobile phase. 5 ml was taken in a separate 50ml volumetric flask and the volume was adjusted to 50 ml with mobile phase to get concentration of 100µg/ml of Bambuterol HCl. 20µl of this solution was injected and chromatogram was obtained

(C) PREPARATION OF MIXED STANDARD SOLUTION:

100.4mg of Bambuterol HCl and 104.6mg Montelukast sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Bambuterol HCl and 100µg/ml of Montelukast sodium . 20 µl of the resulting solution was injected and chromatogram was recorded .

FIXED CHROMATOGRAPHIC CONDITIONS:

INSTRUMENT	: Shimadzu liquid chromatograph LC-20 AT VP
COLUMN	: C18
WAVELENGTH	: 230 nm
TEMPERATURE	: Ambient temperature.
FLOW RATE	: 2ml/min
INJECTION VOLUME	: 20µl.
MOBILE PHASE	**:** Buffer (Potassium di hydrogen phosphate pH:6) acetonitrile. (60:40), finally add 5%v/v THF

RETENTION TIME **:** 4.270 min for Bambuterol HCl , 7.430 min

for Montelukast sodium

QUANTITATIVE DETERMINATION OF THE DRUGS BY USING THE DEVELOPED METHOD

Sample : Montek-plus

Label Claim : Montelukast sodium – 10mg

Bambuterol HCl - 10mg

METHOD:

Twenty tablets were weighed and powdered. 570.2mg sample tablet MONTEK-PLUS (equivalent to 104.6 mg of Montelukast sodium and 100.4 mg Bambuterol HCl) was taken into 100ml dried volumetric flask. The powder was first dissolved in 5ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Montelukast sodium and 100µg/ml of Bambuterol HCl. 20µl of the solution was injected and the chromatogram obtained.

The amount of Montelukast sodium and Bambuterol HCl present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table-1

Amount of drug present in the tablet:

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight}$$

$$\textbf{Percentage purity} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

Table-1

Quantitative Estimation

S. No.	Brand Name	Content	Label Claim (mg)	Peak area	Amount present (mg)	Percent Purity% w/v
1.	MONTEK-PLUS	Bambuterol HCl	10mg	2631.467	10.022	100.42%
		Montelukast sodium	10mg	2731.266	10.025	100.45%

Acceptance critearia: 98-102%w/v

ASSAY FOR BAMBUTEROL HCL:

Amount Present =

$$\frac{2455.716}{2455.686} \times \frac{100.4X50X50}{1000X570.2X5} \times \frac{99.68}{100} \times 114.2=10.022 \text{ mg}$$

% Label Claim =

$$\frac{10.022}{10} \times 100 = 100.22\ \%$$

ASSAY FOR MONTELUKAST SODIUM:

Amount Present =

$$\frac{2649.254}{2656.278} \times \frac{104.6X50X50}{1000X570.2X5} \times \frac{99.54}{100} \times 114.2=10.025 \text{ mg}$$

% Label Claim =

$$\frac{10.025}{10} \times 100 = 100.25\ \%$$

VALIDATION

Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Design of experiment:

Typical analytical parameters used in assay validation are,

- Specificity
- Linearity and range
- Limit of quantification
- Limit of detection
- Accuracy
- Precision
    - System precision
    - Method precision
- Robustness
- Ruggedness
- System suitability studies
    - Resolution
    - Number of theoretical plates
    - The tailing factor.

SPECIFICITY

The specificity of an analytical method is its ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix.

Determination:

The specificity of the analytical method was determined by injecting the placebo solution under the same experimental conditions as the assay.

Preparation of placebo:

Placebo is prepared by mixing all the excipients without active ingredients.

Procedure:

- 100mg placebo was accurately weighed and transferred into a 25ml volumetric flask and the volume was made to 25ml with the mobile phase. The solution was filtered through Millipore filter paper and degassed. 20µl of this solution was injected and chromatogram was recorded .
- 100.4mg of Bambuterol HCl and 104.6mg Montelukast sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and it was sonicated. Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and
-

the volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Bambuterol HCl and 100µg/ml of Montelukast sodium.

- To this solution 100mg of placebo was added and it was sonicated ,filtered through a Millipore filter paper. 20 µl of the resulting solution was injected and chromatogram was recorded.The mixed standard solution was also injected without placebo and it was recorded and the reports are shown in Table-9 &10

Table-9

Specificity for Bambuterol HCl

S.No.	Sample	Area obtained
1.	Standarad	2455.686
2.	Standard+Placebo	2454.420
3.	Placebo	0

Table-10

Specificity for Montelukast sodium

S.No.	Sample	Area obtained
1.	Standarad	2656.278
2.	Standard+Placebo	2657.760
3.	Placebo	0

LINEARITY AND RANGE:

Linearity of an analytical method is its ability to elicit test result that are directly proportional to the concentration of analyte in samples within a given range.

Determination:

The linearity of the analytical method was determined by mathematical treatment of test result obtained by analysis of samples with analyte concentrations across the claimed range. Area was plotted graphically as a function of analyte concentration. Percentage curve fitting was calculated.

Method:

Preparation of mixed standard stock solution

Accurately weighed 100.4mg of Bambuterol HCl and 104.6mg Montelukast sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and then the volume was adjusted to 100ml with mobile phase. From the From the resulting solution, 4, 4.5, 5, 5.5, 6ml were transferred into 5 different 50ml volumetric flask. The volume was made with mobile phase to get a final concentration of 80.32,90.36,100.4,110.44,120.48 µg/ml of Bambuterol HCl and 83.68, 94.14, 104.6, 115.06, 125.52 µg/ml of Montelukast sodium. 20µl of the resulting solution was injected and chromatogram was recorded.

Acceptance Criteria

- Correlation coefficient should not be less than 0.99

The linearity datas and analytical performance parameters of Bambuterol HCl and Montelukast sodium are shown in Table-11- 13 .

Table-11

LINEARITY DATA

Bambuterol HCl

S.No.	Concentration(µg/ml)	Peak Area
1.	80.32	1973.618
2.	90.36	2221.115
3.	100.4	2467.211
4.	110.44	2716.779
5.	120.48	2955.656

Table-12

LINEARITY DATA

Montelukast sodium

S.No.	Concentration(µg/ml)	Peak Area
1.	83.68	2126.738
2.	94.14	2398.281
3.	104.60	2663.350
4.	115.06	2926.429
5.	125.52	3196.037

Table-13

ANALYTICAL PERFORMANCE PARAMETERS

S. no.	Drug name	Linear dynamic range(µ/ml)	Correlation coefficient	Slope	Intercept
1.	Bambuterol HCl	(80.32-120.48)	0.999	53.71	82.615
2.	Montelukast sodium	(83.68-125.52)	0.999	25.494	-4.505

ACCURACY

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte.

Determination:

The accuracy of the analytical method was determined by applying the method to the analysed samples to which known amounts of analyte had been added. The accuracy was calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance criteria:

Percentage recovery should be within 98-102%

PROCEDURE:

Mixed standard stock solution 5ml and sample stock solution 5ml were mixed together in 50 ml volumetric flask and the volume was made upto 50ml with mobile phase to get 100% range. Similarly 80% and 120% range was prepared. 20µl of this solution was injected three times and chromatograms were shown in the following graphs and values in table 14 and 15 .

Table-14

Recovery Study of Bambuterol HCl

S.No.	RANGE	Area obtained	Amount Recovered(mg)	% Recovery
1.	80%	1979.529	10.088	100.88
		1976.637	10.073	100.73
		1975.437	10.066	100.66
2	100%	2447.370	9.97	99.77
		2446.793	9.97	99.77
		2443.684	9.96	99.62
3	120%	2941.021	9.99	99.91
		2951.066	10.02	100.25
		2944.075	10.002	100.02

Table-15

Recovery Study of Montelukast sodium

S.No.	RANGE	Area obtained	Amount Recovered(mg)	% Recovery
1.	80%	2126.532	10.12	101.23
		2133.350	10.15	101.56
		2147.210	10.22	102.22
2	100%	2657.783	10.11	101.17
		2655.368	10.113	101.13
		2654.627	10.110	101.10
3	120%	3176.470	10.08	100.81
		3198.139	10.15	101.50
		3186.244	10.11	101.12

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

Determination:

The precision of the analytical method was determined by assaying sufficient number of sample and relative standard deviation was calculated.

The precision of the instrument was determined by assaying the samples consecutively, number of time and relative standard deviation was calculated.

Acceptance Criteria:

The relative standard deviation should be with in 2%

SYSTEM PRECISION :

Accurately weighed 100.4mg of Bambuterol HCl and 104.6mg Montelukast sodium sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF and then the volume was adjusted to 100ml with mobile phase. From the resulting solution 5ml was transferred into 50ml volumetric flask. The volume was made up with mobile phase to 50ml.

Method:

The system precision was evaluated by measuring 6 successive injection of 20µl of standard solution. The peak response were measured from the following chromatogram and system precision data area shown in Table-15&16.

METHOD PRECISION:

Procedure

Twenty tablets were weighed and powdered. 570.2mg sample tablet MONTEK-PLUS(equivalent to 104.6mg of Montelukast sodium and 100.4mg Bambuterol HCl) was taken into 100ml dried volumetric flask. The powder was first dissolved in 10ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Montelukast sodium and 100µg/ml of Bambuterol HCl. 20µl of the solution was injected and the chromatogram obtained is shown in following graph.

The amount of Montelukast sodium and Bambuterol HCl present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table-17-19

Table-15

System Precision data

S.No.	Area of Bambuterol HCl	Area of Montelukast sodium
1.	2441.405	2639.803
2.	2446.671	2670.187
3.	2453.085	2658.527
4.	2458.308	2668.277
5.	2445.602	2644.152
6.	2455.507	2657.305
MEAN	2450.097	2656.377
S.D	2.9196	5.5226
%RSD	0.119	0.002

Table-16

Relative Standard Deviation	Bambuterol HCl	Montelukast sodium	Acceptance Criteria
	0.119	0.002	2%

Table-17

Method Precision 0f Bambuterol HCl

S.No.	Area Obtained	Assay value in(mg)	% Label claim w/v
1.	2455.716	10.0698	100.6%
2.	2439.994	9.9837	99.83%
3.	2450.901	10.002	100.02%
4.	2442.650	9.94	99.4%
5.	2457.210	10.08	100.8%
6.	2446.320	9.97	99.7%
	MEAN		2448.798
	STANDARD DEVIATION		3.1272
	RELATIVE STANDARD DEVIAION		0.0012

Table-18

Method Precision 0f Montelukast sodium

S.No.	Area Obtained	Assay value in(mg)	% Label claim w/v
1.	2649.254	10.046	100.4%
2.	2652.369	9.944	99.4%
3.	2629.900	99.903	99.03%
4.	2632.810	9.878	98.78%
5.	2656.440	10.05	100.5%
6.	2645.434	9.96	99.6%
	MEAN		2644.368
	STANDARD DEVIATION		4.8069
	RELATIVE STANDARD DEVIAION		0.0018

Table-19

Relative Standard Deviation	Bambuterol HCl	Montelukast sodium	Acceptance Criteria
	0.0012	0.0018	2%

Limit of detection (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conditions. The detection limit is usually expressed as the concentration of analyte.

It is given by

$$L.O.D = \frac{3.3 \times \sigma}{m}$$

σ = standard deviation of the response

m= slope of the calibration curve

TABLE-20

LIMIT OF DETECTION

DRUG	STANDARD DEVIATION	SLOPE	L.O.Dµg/ml
BAMBUTEROL HCL	15.874	24.499	2.1382
MONTELUKAST SODIUM	16.538	25.494	2.1407

Limit of Quantitation:

The Quantitation limit of an analytical procedure is the lowest amount of analyte which can be Quantitatively determined with suitable Precision and Accuracy.

It is given by

$$L.O.Q = \frac{10x\sigma}{m}$$

σ = standard deviation of the response

m= slope of the calibration curve

TABLE-21

LIMIT OF QUANTITATION

DRUG	STANDARD DEVIATION	SLOPE	L.O.Q µg/ml
BAMBUTEROL HCL	15.874	24.499	6.4794
MONTELUKAST SODIUM	16.538	25.494	6.4870

RUGGEDNESS

The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under a variety of normal test condition, such as different laboratories, different analyst, different instruments, different lots of reagents, different elapsed assay times, different assay temperature, different days, etc.

Ruggedness is normally expressed as the lack of influence on test result of operational and environmental variables of the analytical method.

Determination:

The ruggedness of an analytical method was determined by analysis of aliquots from homogeneous lots by different analysts using operational and environmental conditions that may differ but were still with in the specified parameters of the assay. The degree of reproducibility of test result was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions to obtain a measure of the ruggedness of analytical method.

The assay of BAMBUTEROL HCL and MONTELUKAST SODIUM were performed in different conditions like different analyst on different days.

Method:

The standard and sample solutions were prepared by different analysts on different days and the resulting solution were injected and chromatograms are recorded and shown in

following graphs and ruggedness of the method and report of Bambuterol HCl and Montelukast sodium are shown in Table 22.

Table-22

RUGGEDNESS

Analyst	Date	Amount Found		%purity	
		Bambuterol HCl mg	Montelukast sodium mg	Bambuterol HCl	Montelukast sodium
I	17/12/2009	9.968	10.012	99.68	100.12
II	18/12/2009	9.95	10.042	99.5	100.7

ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination:

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameter of the assay for example change in physical parameters like flow rate and lambda max.

Method:

Standard solution preparation:

100.4mg of Bambuterol HCl and 104.6mg Montelukast sodium was transferred into a 100ml dried volumetric flask. The compounds were first dissolved in 10ml of THF.Then the volume was adjusted to 100ml with mobile phase. From the stock solution 5ml was transferred to a 50ml volumetric flask and the volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Bambuterol HCl and 100µg/ml of Montelukast sodium.

Sample preparation:

Twenty tablets were weighed and powdered. 570.2mg sample tablet MONTEK-PLUS(equivalent to 104.6mg of Montelukast sodium and 100.4mg Bambuterol HCl) was taken into 100ml dried volumetric flask. The powder was first dissolved in 10ml of THF and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 100µg/ml of Montelukast sodium and 100µg/ml of Bambuterol HCl. 20µl of the solution was injected and the chromatogram obtained is shown in following graphs.

The amount of Montelukast sodium and Bambuterol HCl present in the tablet formulation was calculated by comparing the peak area of the standard and reports are given in Table-23-30

Table 23

Chromatographic condition

flow rate	2ml/min
Column	C_{18}
Wave length	230nm
Temperature	Ambient 25^{o}c
Injection Volume	20µl

Table 24

Change in Organic phase +10%

S.No.	Drug	Standard Area	Sample Area	% Purity w/v
1.	Bambuterol HCl	2165.169	2188.211	102.7
2.	Montelukast sodium	1503.266	1507.421	100.6

Table 25

Chromatographic condition:-

Change in flow rate	2ml/min
Column	C_{18}
Wave length	230nm
Temperature	Ambient25^{o}c
Injection Volume	20µl

Table 26

Change in organic phase -10%

S.No.	Drug	Standard Area	Sample Area	% Purity w/v
1.	Bambuterol HCl	2509.217	2501.504	99.80
2.	Montelukast sodium	1586.911	1582.612	100.1

Table 27

Chromatographic condition: - change in Lambda max 230 nm

flow rate	2.0 ml/min
Column	C_{18}
Wave length	230nm
Temperature	Ambient25^{o}c
Injection Volume	20µl

Table 28

Change in Lambda max 232 nm

S.No.	Drug	Standard Area	Sample Area	% Purity w/v
1.	Bambuterol HCl	2446.667	2443.332	99.9
2.	Montelukast sodium	2646.951	2645.612	100.4

Table 29

Chromatographic condition:- change in Lambda max 228 nm

flow rate	2.0 ml/min
Column	C_{18}
Wave length	267nm
Temperature	Ambient25^{o}c
Injection Volume	20µl

Table 30

Change in Lambda max 228 nm

S.No.	Drug	Standard Area	Sample Area	% Purity w/v
1.	Bambuterol HCl	2097.491	2095.237	100.01
2.	Montelukast sodium	2455.953	2456.413	100.4

SYSTEM SUITABILITY PARAMETERS

System suitability testing is an integral part of many analytical procedures. The test is based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Method:

A solution of 100.1 µg/ml Bambuterol HCl and 104.6 µg/ml were prepared by diluting with mobile phase and same was injected and a chromatogram was recorded and they are shown in the following graph and system suitability report are shown in the following Table-31

Table 31

System suitability parameters

S.No.	Parameters	Bambuterol HCl	Montelukast sodium
1.	Theoretical plates	10133	12505
2.	Tailing factor	1.074	1.023
3.	Resolution	14.520	

UV SPECTROPHOTOMETRIC SIMULTANEOUS EQUATION METHOD OF MONTELUKAST SODIUM AND BAMBUTEROL HCl IN COMBINED TABLET DOSAGE FORM

PRINCIPLE:

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other. It may be possible to determine the quantity of both drugs by the technique of simultaneous equation (or) Vierodt's method.

Criteria for obtaining maximum precision, based upon absorbance ratios have been suggested that place limits on the relative concentrations of the component of the mixture.

$$\frac{A_2 / A_1}{ax_2 / ax_1} \text{ and } \frac{ay_2 / ay_1}{A_2 / A_1}$$

Where ax_1, ax_2 = Absorptivities of X at λ_1 and λ_2

ay_1, ay_2 = Absorptivities of X at λ_1 and λ_2

A_1, A_2 = Absorbances of the diluted sample at λ_1 and λ_2.

The ratio should lie outside the range of 0.1 – 2.0 for the precise determination of (Y and X) two drugs respectively.

These criteria are satisfactory only when the λ_{max} of the two components is reasonably dissimilar. The additional criteria includes that two components do not interact chemically,

there by negating the initial assumption that the total absorbance is the sum of the individual absorbances.

MATERIALS

Market Sample: Montek Plus

LABEL CLAIM:

Montelukast sodium -10 mg

Bambuterol Hcl -10 mg

Equipments Used:

- ATCO Balance
- SHIMADZU UV - & spectrophotometer double beam digital UV-1700

Solvent Used:

- Methanol AR

FIXATION OF VARIOUS PARAMETERS (Montelukast Sodium)

λ_{max}

The wavelength at which maximum absorption takes is place called λ_{max}

Determination of Absorption Maximum (λ_{max}) for Montelukast Sodium

Procedure:

100mg of authentic **Montelukast Sodium** sample was accurately weighed and transferred to 100ml volumetric flask and 10ml methanol was added, dissolved and the volume was made upto 100ml with methanol.

10ml of this stock solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol. From this 10ml of solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

The absorbance of solution was measured against solvent blank in UV-region of 200-400nm. The λ_{max} was found to be 280nm.

BEER'S LAW PLOT FOR MONTELUKAST SODIUM

PROCEDURE

100mg of authentic **Montelukast Sodium** sample was accurately weighed and transferred to 100ml volumetric flask and 10ml methanol was added, dissolved and the volume was made upto 100ml with methanol.

10ml of this stock solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

From this aliquots of 4ml, 8ml, 12ml, 16ml, 20ml, 24ml was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 280nm against a reagent blank. The readings are presented in Table-32 and the following graph A.

Table – 32

DATA FOR BEER'S LAW PLOT OF MONTELUKAST SODIUM

(Linearity)

S.No.	Concentration µg/ml	Absorption
1	4	0.098
2	8	0.198
3	12	0.299
4	16	0.401
5	20	0.502
6	24	0.602

Linearity Co-efficient (γ) = 0.995

SLope (m) = 0.0252

Intercept(c) =

DEVIATIONS FROM BEER'S LAW

For the drug **Montelukast sodium** maximum deviation was found in the Concentration range above 36µg/ml. The readings are presented in

Table -33 and the following graph B.

Table -33

DATA FOR DEVIATIONS FORM BEER'S LAW PLOT FOR

MONTELUKAST SODIUM

S.No.	Concentration µg/ml	Absorbance
1.	4	0.098
2.	8	0.198
3.	12	0.299
4.	16	0.401
5.	20	0.502
6.	24	0.602
7.	28	0.712
8.	32	0.828
9.	36	0.843

Determination of Absorption Maximum (λ_{max}) for Bambuterol Hcl

The literature survey shows that there is no specific λ_{max} for bambuterol Hcl.

I also confirmed it. But some studies was carried out by taking 210-215nm as a working standard. So I carried out this work by taking 212 nm as a working standard for bambuterol Hcl.

BEER'S LAW PLOT FOR BAMBUTEROL HCl

PROCEDURE

100mg of authentic bambuterol Hcl. sample was accurately weighed and transferred to 100ml volumetric flask and methanol was added, dissolved and the volume was made upto 100ml with methanol.

10ml of this stock solution was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol.

From this aliquots of 4ml, 8ml, 12ml, 16ml, 20ml, 24ml was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol. The absorbance of each solution was found out at 212nm against a reagent blank. The readings are presented in Table-34 and the following graph c.

Table – 34

DATA FOR BEER'S LAW PLOT OF BAMBUTEROL HCl

(Linearity)

S.No.	Concentration µg/ml	Absorption
1	4	0.212
2	8	0.418
3	12	0.632
4	16	0.851
5	20	1.070
6	24	1.285

Linearity Co-efficient (γ) = 0.993

SLope (m) = 0.0538

Intercept(c) =

DEVIATIONS FROM BEER'S LAW

For the drug **Bambuterol HCl** maximum deviation was found in the Concentration range above 28µg/ml. The readings are presented in

Table -35 and the following graph D.

Table -35

DATA FOR DEVIATIONS FROM BEER'S LAW PLOT FOR

BAMBUTEROL HCl

S.No.	Concentration µg/ml	Absorbance
1.	4	0.212
2.	8	0.418
3.	12	0.632
4.	16	0.851
5.	20	1.070
6.	24	1.285
7.	28	1.301
8.	32	1.324

Preparation of sample solution

Ten tablets are weighed and average weight was calculated. The tablets are ground to a fine powder. A powder equivalent to 10mg of Montelukast Sodium and 10mg of Bambuterol Hcl was accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made upto 100ml with methanol. This solution was filtered through whatmann filter paper.

From this 10ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol. From this aliquots any one concentration (4ml or 8ml, 12ml, 16ml, 20ml, 24ml) was pipetted out in to separate 100ml volumetric flask. Then the volume was made upto 100ml with methanol

The absorbance of each solution was found out at 280nm (λ_{max} of Montelukast Sodium) and 212nm (Working standard for Bambuterol Hcl) against a reagent blank.

The analysis values are given in Table-36

Calculation:

λ_1 = 280nm (λ_{max} of Montelukast Sodium)

λ_2 = 212nm (Working standard for Bambuterol Hcl)

X – Montelukast Sodium

Y – Bambuterol Hcl

ax_1 and ax_2 – Absorptivities of Montelukast Sodium at λ_1 and λ_2

ay_1 and ay_2 – Absorptivities of Bambuterol Hcl at λ_1 and λ_2

Cx and Cy – Concentration of Montelukast Sodium and Bambuterol Hcl

(Sample) in grams per 100ml

A1 and A2 – Absorbance of sample at λ_1 and λ_2

$$\text{Absorptivity (a)} = A/bc = \frac{\text{Absorbance}}{\text{b x concentration of substance}}$$

Determination of Cx and Cy

$$Cx = \frac{A_2ay_1 - A_1ay_2}{ax_2\,ay_1 - ax_1\,ay_2}$$

$$Cy = \frac{A_1ax_2 - A_2ax_1}{ax_2\,ay_1 - ax_1\,ay_2}$$

Table36

Absorbance values for standard and sample

Wavelength	Montelukast Sodium	Bambuterol Hcl	**Sample**
280 λ_1 Montelukast Sodium	0.502	0.145	0.271
212 λ_2 Bambuterol Hcl	0.091	0.851	0.0472

Table-36a

Absorptivity values for Montelukast Sodium and Bambuterol Hcl

Parameter	**Absorptivity at 280nm**		**Absorptivity at 212nm**	
	Montelukast Sodium	**Bambuterol Hcl**	**Montelukast Sodium**	**Bambuterol Hcl**
*Mean	0.35	0.1046	0.0755	0.7446
SD	0.6633	0.3577	0.3924	0.2366

* Absorptivity values are the mean of six determinations. S.D. is standard deviation. ax1 and ax2 are absorptivities of Montelukast Sodium at 280 nm, and 212nm, respectively; ay1 and ay2 are absorptivities of Bambuterol HCl at 280nm and 212nm respectively.

CRITERIA FOR OBTAINING MAXIMUM PRECISION

$(A_2 / A_1)/ (ax_2 / ax_1)$ and $(ay_2 / ay_1)/ (A_2 / A_1)$

was found to be 0.9611 and 0.9781 respectively. This ratio should lie outside the range 0.1-2.0 for the precise determination.

Table-36b

Analysis data of tablet formulations

Parameters	**UV-spectrophotometry**	
	Montelukast Sodium	**Bambuterol HCl**
Label Claim	10mg	10mg
Amount found	10.11 mg	9.95 mg
*% Drug content	101.1%	99.5%
S.D.	1.8172	0.18289
% R.S.D	1.8152	0.1827

- value for % Drug content are mean of five estimations

RECOVERY STUDIES

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120% level. From the total amount of drug found the percentage recovery was calculated. The results are reported in Table-37

Table 37

Recovery studies

Montelukast Sodium

Range	*Recovery	% R.S.D
80%	101.23	0.8020
100%	101.13	0.6435
120%	100.81	0.4765

Bambuteol HCl

Range	*Recovery	% R.S.D
80%	100.66	0.1823
100%	99.77	0.1000
120%	99.91	0.1674

*Recovery is the mean of three estimations

RESULTS AND DISCUSSION

Montelukast sodium and Bambuterol HCl

UV spectrophotometry by simultaneous equation method and reverse phase high performance liquid chromatography were developed for analysing Montelukast sodium and Bambuterol HCl in combined tablet dosage form.

For UV spectrophotometry linearity was obtained in the concentration range of 4 to 32 µg/ml for Montelukast sodium and 4 to 28 µg/ml for Bambuterol HCl. In quantitative determination the % Drug content was found to be 101.1% and 99.5% for Montelukast sodium and Bambuterol HCl respectively. Recovery experiments were performed and it was within 98 – 102%, the percentage relative standard deviation were found to be <2% which shows high precision and accuracy of the method.

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of eluted compounds. Initially various mobile phase were tried, to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, etc). The system with buffer (Potassium hydrogen phosphate pH6): Acetonitrile : methanol (60:40v/v) with 2 ml/min flow rate is quite robust. The optimum wavelength for detection was 230nm at which better detector response for drugs was obtained. The average retention times for 4.28 min for Bambuterol HCl ,7.4 min for Montelukast sodium respectively.

According to USP system suitability test are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To

ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution. The parameters are shown in table 31.

The calibration curve was found to be linear for both Bambuterol HCl & Montelukast sodium.

The low values of % RSD indicate the method is precise and accurate. The developed method was very specific without the interference of excipients.

The percentage purity was 99.97% and 100.8% for Bambuterol HCl and Montelukast sodium respectively.

The mean recoveries were found to be in the range of 98% to 102%.

Limit of detection for Bambuterol HCl and Montelukast sodium was found to be 2.1382 µg/ml & 2.1407 µg/ml respectively.

Limit of quantitation for Bambuterol HCl and Montelukast sodium was found to be 6.4794 µg/ml & 6.4870 µg/ml respectively.

Robustness of the proposed method was determined by changing the wavelength and flow rate.

Ruggedness of proposed method was determined by analysis of aliquots from homogenous slot by different analyst in different days using similar operational environmental condition. The results were within 98-102%.

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